transmembrane proteins to avoid denaturation. This new capability to separate membrane-bound species near native conditions based on affinity for certain lipid phases can be used to identify intrinsic membrane raft residents and to characterize how post-translational modifications shift the affinity of analogs to a particular lipid phase.

143-Plat
Multiplexed Microfluidic Device for Bilayer Experimentation and Drug Screening Assays on Membrane Proteins
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Membrane proteins represent >50% of the targets for the development of new drugs. However, to date no appropriate platform is available for high throughput drug screening assays on these proteins. In that context, we developed a 1-plex microfluidic device for experimentation on BLMs (bilayer lipid membrane) and membrane proteins.[1] Furthermore, we have proposed a novel methodology to prepare membranes in a closed environment, the ''lipid-plug thinning'' technique, and we have validated our device for the detection of single pore-forming species.
Here, we report the multiplexing of the platform for parallel experimentation on a series of independent BLMs. The same 3-layer glass-Teflon-glass structure is employed, where the glass substrates house microchannels and the Teflon layer microfabricated apertures (15-40 μm Ø) for BLM formation. Two potential designs are investigated. In the first design, a series of independent 1-plex devices is placed in parallel. The second design resembles the structure of a fishbone, with one common channel in one glass substrate and a series of independent channels in the second glass substrate to reduce the number of reservoirs. Bilayers are formed in the multiplexed devices with a slightly modified approach where the lipid solution is introduced in one channel only, while buffer is present in the other one. Bilayer formation is successful (~75% yield) for both designs, and BLMs exhibit good properties in terms of seal resistance (>10 GΩ), and capacitance (1.35-5.01 pF for 30-μm apertures).
We are currently comparing the two designs for both automation of the membrane formation and simultaneous electrical measurements on independent BLMs. Thereafter, we will conduct experiments on pore-forming species and membrane properties using the in-house developed gramicidin-based assay.[2]
1. Stimberg et al., Proceedings MicroTAS 2010 & 2011.
2. Stimberg et al., Small, submitted.

144-Plat
Patterned Substrates for the Study of Axonal Differentiation and Neuronal Response to Smooth Microtopography
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The development of two and three-dimensional patterned substrates provides new ways to study neuronal behavior. The creation of two-dimensional (2D) patterned surfaces through the use of photolithography, microcontact printing, and self-assembled monolayer (SAM) chemistry allows for the study of axonal differentiation. Through the use of these methods, we have created starburst patterns to which E18 mouse hippocampal neurons are confined. Utilizing immunohistochemistry to specifically stain for the tau protein, predominantly localized along microtubules in the axon, we have found that neurite differentiation is not a predetermined process; rather, it is environmentally determined. The compilation of statistical data has shown that a mere 20μm difference in the distance a neurite is allowed to grow will determine polarization.
Grayscale photolithography and solvent-assisted molding (SAMo) allow us to create smooth microtopography that mimics the microtopography encountered in vivo. We have fabricated continuous three-dimensional (3D) wave patterns, varying height as well as the peak-to-peak distance, to the study of neuronal behavior in response to smooth variations in microenvironment. This will provide insights into the limitations a neuron may experience during pathfinding in vivo.

145-Plat
Red Blood Cell Sickling During Oxygen Cycles in a Microdroplet Device
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We have developed a novel microfluidic device to study repetitive sickling on individual red blood cells by replicating the physiological oxygen cycling of the vascular circulatory system (Abbyad et al., Lab Chip, 2011, 11, 813). A small number of red blood cells from sickle cell patients are encapsulated in an array of aqueous microdroplets. These microdroplets are anchored and arranged in a 2-dimensional array against the flow of the carrier oil. Precise spatial and temporal changes in oxygen concentration are obtained through gas exchange with the inert oil flowing outside the droplets. By oscillating the oxygen concentration, cycles of sickling and desickling of individual red blood cells are observed in real-time. Polarization microscopy allows for the sensitive detection of intracellular hemoglobin fibers. We observed small residual intracellular hemoglobin fibers that remain even in oxygenated conditions. Since the content of droplets in the array can be controlled, active molecules at different concentrations as well as control droplets can be measured side-by-side as they are exposed to the same environmental conditions. This was used to measure cell sickling in the presence and the absence of the anti-sickling agent glyceraldehyde. The cumulative impact of repeated sickling, such as membrane damage and cell dehydration, is believed to be central to disease pathology. We are now studying phosphatidylserine outer leaflet externalization and cell dehydration as a function of deoxygenation cycle.

146-Plat
Body Mechanics Regulate the Force Threshold for Gentle Touch Sensation in the Nematode C. Elegans
Bryan C. Petzold[1], Sung-Jin Park[1], Miriam B. Goodman[2], Beth L. Pruitt[1].
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Touch is among the least understood of our senses despite its importance in our daily lives. In the model organism *C. elegans*, gentle touch is detected by six touch receptor neurons situated in the outer shell of the animal. Force applied to the body is filtered by the outer shell (cuticle, hypodermis and body wall muscles) of the body, locally straining nearby touch receptor neuron(s) and opening mechanically-gated DEG/ENaC channels through an unknown mechanism. Previously we developed a piezoresistive cantilever force clamp system capable of applying calibrated loads to moving *C. elegans* [S.J. Park et al., Rev Sci Instr (2011), 82:043703] and showed that wild-type (N2) animals respond to forces of only 100s of nN. Further, we showed that the outer shell of the animal dominates overall body stiffness [S.J. Park et al., PNAS (2007), 104:17376]. Since the touch receptor neurons lie within the outer shell and the outer shell controls the overall mechanics of the body, we hypothesized that the force threshold for gentle touch avoidance is regulated by body stiffness. Building on our prior work showing that body stiffness can be reversibly modulated with optogenetically-induced changes in body wall muscle tone [B.C. Petzold et al., Biophys J (2011), 100:1977], we measured the force threshold for behavioral response while modulating body stiffness with Channelrhodopsin-2. In animals with hypercontracted muscles and elevated body stiffness, we found that larger forces were generally required to elicit a touch avoidance response. These findings suggest that body mechanics play an important role in filtering applied loads to the touch receptor neurons, ultimately modulating the force sensitivity of the animal, and imply that skin plays a critical role in touch sensation in both *C. elegans* and higher organisms.

Symposium: Temperature Regulation of Channels

147-Symp
Regulation of the Cold Sensor TRPM8 Channels
Tibor Rohacs.
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Transient Receptor Potential Melastatin 8 (TRPM8), channels are well established sensors of environmental cold temperatures. They can also activated by chemical agonist, such as menthol and icilin. Activation of these channels requires the presence of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. This presentation will discuss the role this lipid plays in the regulation of TRPM8 channels upon cold and menthol activation.

148-Symp
Modular Thermal Sensors in Temperature-Gated TRP Channels
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Physiology and Biophysics, SUNY-Buffalo, Buffalo, NY, USA.
A group of ion channels in the TRP family, the so-called thermal TRP channels, exhibit unprecedentedly strong temperature dependence, some of which reach Q10 > 100 as compared to 2-3 for typical enzymatic reactions. The strong thermal sensitivity of these channels arises because their gating involves large

enthalpy changes between closed and open. To quantify the energetics of gating, we applied optically generated submillisecond temperature jumps to directly measure the temperature-dependent activation kinetics. The data show that the opening of TRPV1, for example, is accompanied with an enthalpy change of ~100 kcal/mol, five times the energetic changes in voltage- or ligand-dependent gating. To gain insights on the energetic source, we analyzed single-channel and macroscopic kinetics of temperature-dependent gating, both showing that temperature has localized effects on specific gating components. Furthermore, the perturbation of membrane compositions, which altered the physical properties of lipids such as temperature-dependent phase transitions, did not abolish temperature activation of the channel. Thus the thermal sensitivity of the channel appears to be intrinsic to the channel itself, most likely arising from a specific protein domain rather than integration of global thermal effects. Using systematic chimeric analysis, we uncovered a proximal N-terminal region to be crucial for temperature sensing in heat-activated TRPVs. Changing this region both successfully transferred thermal sensitivity to temperature-insensitive isoforms and profoundly altered thermal sensing in temperature-sensitive wild-type channels. Swapping other domains including the whole transmembrane core, the C terminus, and the rest of the N terminus had little effect on the large enthalpy of gating. These results support that thermal TRP channels contain modular thermal sensors for their activation by temperature.

149-Symp
Heat Activation of TRPV Channels
Jie Zheng.
UC Davis, Davis, CA, USA.
Activation of TRPV1-4 channels is exquisitely heat-sensitive due to large and balanced changes in entropy and enthalpy. Identification of channel structures that underlie these changes is thus the prerequisite for understanding the molecular mechanism of heat activation. We approach this goal through a combination of methods. We use improved heating methods to accurately control temperature-dependent activation of wildtype channel and mutants which exhibit altered gating behavior, use structural analysis to guide our search for critical domains, and use site-directed fluorescence recordings to monitor conformational changes. For capsaicin receptor TRPV1, heat activation appears to be carried out by the central pore domain, while capsaicin is known to activate the channel by binding to the peripheral S2-S4 region. Structurally and mechanistically separated heat and agonist activation pathways suggest the possibility to selectively manipulate each process in this pain sensor for pharmaceutical purposes. The heat activation process appears to be preserved among TRPV1-3 channels.

150-Symp
Searching for Voltage Sensors in Thermosensitive TRP Channels
Viktorie Vlachova.
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Temperature-activated transient receptor potential (TRP) channels are transmembrane proteins that play important roles in the transduction of thermal and chemical stimuli. In addition to the thermal and chemical cues, these channels can be activated by depolarizing the cell membrane, but the molecular basis of this regulation is unclear. The transmembrane part of the tetrameric TRP channels is structurally related to the voltage-gated K^+ channels in which the conserved charged residues within the fourth transmembrane region (S4) constitute part of a voltage sensor. Compared to these channels, the voltage-dependence of TRP channels is very weak, exhibiting the apparent number of gating charges of less than 1 versus ~12 in K^+ channels, and their putative voltage-sensing domains most likely lie outside the S4 because some of the TRPs completely lack positively charged residues in this region. In the present study, we will attempt to explore the functional roles of selected conserved charged residues which mutations specifically alter the voltage sensitivity of the vanilloid (TRPV) and ankyrin (TRPA1) channels. In particular, we will show that potential voltage-sensing residues in S4 and the S4-S5 linker, when specifically mutated, alter the functionality of these channels with respect to voltage, but also to temperature, agonist, and/or their interactions. Surprisingly, also a single amino acid substitution in the C-terminus led to substantial alterations in the voltage-dependent gating of TRPA1. In summary, the charged residues in S4, the S4-S5 linker, and in the C-terminus contribute to voltage sensing in some thermosensitive TRP channels and, despite their highly conserved nature, regulate the voltage and chemical gating in various related TRP channels in different ways. (Supported by GACR 305/09/0081)

Symposium: Spatial Organization in Prokaryotic Cells: Quantitative Measurements to Quantitative Models

151-Symp
Designing Intracellular Organization for Optimization of Sustainability
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Biology presents us with an array of design principles. From studies of both simple and more complex systems, we understand some of the fundamentals of how Nature works. We are interested in using the foundations of biology to engineer cells in a logical and predictable way to perform certain functions. By necessity, the predictable engineering of biology requires knowledge of quantitative behavior of individual cells and communities and the ability to construct reliable models. By building and analyzing synthetic systems, we learn more about the fundamentals of biological design as well as engineer useful living devices with myriad applications. For example, we are interested in building cells that can perform specific tasks, such as remembering past events and thus acting as a biological computer. Moreover, we design cells with predictable biological properties that serve as cell-based sensors, factories for generating useful commodities and improved centers for carbon fixation. We have recently constructed synthetic intracellular protein/RNA structures to increase the efficiency of biological reactions. In doing so, we have made new findings about how cells interact with and impact their environment.

152-Symp
Modeling the Shape and Growth of Bacterial Cells
Ned Wingreen[1], Leon Furchtgott[2], K.C. Huang[3].
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In bacterial cells, the peptidoglycan cell wall is the stress-bearing structure that dictates cell shape. Although many molecular details of the composition and assembly of cell-wall components are known, how the network of peptidoglycan subunits is organized to give the cell shape during normal growth has been relatively unexplored. We have introduced a computational physical model of the bacterial cell wall to complement experimental studies of cell-shape determination, with a particular focus on rod-shaped cells like *Escherichia coli*. First, the model predicts the mechanical response of cell shape to peptidoglycan damage and perturbation. We observe a surprising robustness of cell shape to peptidoglycan defects, helping explain the observed porosity of the cell wall and the ability of cells to grow and maintain their shape even under conditions that limit peptide crosslinking. Interestingly, according to the model, many common bacterial cell shapes can be realized via modest local patterning of peptidoglycan density. Second, we introduced growth processes into the model via insertion of new glycan strands, formation of new peptide crosslinks, and cleavage of old crosslinks (1). The growth model suggests that maintaining a rod shape requires glycan insertion to be insensitive to fluctuations in cell-wall density and stress. Suggestively, in light of the role of MreB in maintaining rod-shaped growth, we find that a simple helical pattern of insertion is sufficient for many-fold elongation without significant loss in rod shape. Finally, we demonstrate that both the length and prestretching of newly inserted strands can regulate cell width. In sum, we show that simple physical rules can allow bacteria to achieve robust, shape-preserving cell-wall growth.
1. Furchtgott L, Wingreen NS, Huang KC. Mechanisms for maintaining cell shape in rod-shaped Gram-negative bacteria. Mol Microbiol. 2011; 81(2):340-53.

153-Symp
A Journey to the Pole: Polar Localization of Proteins in E. coli
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Far from being well-mixed, almost all biological systems exhibit precise spatial and temporal control of protein, mRNA, and DNA localization, demonstrating that cells measure distance and detect proximity with a molecular-scale tool kit. Despite its relatively simple cellular structure and diminutive size, Escherichia coli exhibits a high degree of spatial organization. We examine and quantitatively characterize the phenomena of polar localization at a systems scale by capturing the cell-cycle localization dynamics of all proteins with polar